EFFICIENT MICROBIAL BIOCONVERSION OF BROWN MACROALGAE OBTAINED THROUGH PROFITABLE HIGH-DENSITY SEA CULTIVATION USING MODIFIED MICROBIAL STRAINS TO PRODUCE COMMODITY AND SPECIALTY CHEMICALS: A DEVELOPING BLUE CHEMICAL INDUSTRY IN CHILE

Alvaro Olivera-Nappa, Centre for Biotechnology and Bioengineering – CeBiB, University of Chile, Chile
aolivera@ing.uchile.cl
Javiera Gómez, Centre for Biotechnology and Bioengineering – CeBiB, University of Chile, Chile
Carolina Camus, Centre for Biotechnology and Bioengineering – CeBiB and Centro i~mar, Universidad de Los Lagos, Chile
Alejandro H. Buschmann, Centre for Biotechnology and Bioengineering – CeBiB and Centro i~mar, Universidad de Los Lagos, Chile

Key Words: profitable microbial conversion, brown macroalgae carbohydrates, metabolic engineering, dynamic metabolic models, metabolic potentiality maps.

Plant biomass is considered a promising feedstock for large scale sustainable bio-based green chemistry. However, only the use of agricultural or forestry residues is viable, since they do not compete for land with feed crops and have competitive costs. Moreover, carbohydrate recovery from these sources is always difficult due to their high lignin content. Alternatively, macroalgae are competitive sources of carbohydrate-rich biomass not requiring land or fresh water for its production. *Macrocystis pyrifera* is one of the fastest-growing macroalgal species with high CO_2 fixation efficiency, highly-abundant and accessible carbohydrates. We demonstrated that it can be cultured in temperate seas, yielding 124 ton/Ha/yr, and can be economically profitable at a 10-hectare scale [1,2]. Microbial and enzymatic algal biomass bioprocessing has been also undertaken by our group. We demonstrated the technical feasibility of producing ethanol at a pilot industrial scale by fermenting algal carbohydrates with a genetically modified *Escherichia coli* [3]. However, ethanol production, even with high productivities, was not commercially viable. To make algal biomass bioconversion profitable, we performed a large metabolic engineering and synthetic biology project to discover combinations of metabolic pathways, regulation, carbohydrate sources –algal or not– and alternative bioproducts that maximize microbial efficiency and commercial viability. Using a genome-scale reconstruction of *Saccharomyces cerevisiae*'s metabolism, we demonstrated that redox ratio constraints and the preferential use of NADH or NADPH for alginate metabolism were key for *S. cerevisiae* conversion of alginate:mannitol carbohydrate sources [4]. However, yeast use makes chemical processes technically and economically unfeasible for low value products due to their inability to produce extracellular enzymes for alginate lysis. By means of dynamic metabolic models developed for *E. coli*, we demonstrated that the main metabolic process bottleneck is microbial carbohydrate metabolization and that algal carbohydrate composition is a key determinant of fermentation efficiency. Using a multi-objective optimization strategy focused on microorganism growth, energy levels and redox ratio conservation, we also showed that ethanol production from algal biomass is incompatible with *E. coli*'s metabolism, due to low energetic and redox efficiencies obtained from alginate using host microorganism metabolic pathways. We then used high-performance parallel computing to develop a metabolic potentiality map for *E. coli* in which we explored more than 10.000 possible combinations of metabolic pathways that could be built in our strain to convert brown macroalgae carbohydrates with high efficiency, considering the best combinations of knock-outs and overexpressions to be introduced in *E. coli*'s central metabolic pathways. With this technique, we identified other valuable chemicals, such as succinic, aspartic, gluconic and levulinic acids, and complex aromatic and aliphatic biomolecules can be efficiently produced from *Macrocystis* with specifically modified strains for each product. The bulk of our research fostering algal feedstock production and industrial bioconversion in Chile will be presented in this work.

1. Buschmann, A. H. et al. The Status of Kelp Exploitation and Marine Agronomy, with Emphasis on Macrocystis pyrifera, in Chile. *Advances in Botanical Research* 71, 161–188 (2014).
2. Camus, C., Infante, J. & Buschmann, A. H. Overview of 3 year precommercial seafarming of Macrocystis pyrifera along the Chilean coast. *Reviews in Aquaculture* 10, 543–559 (2018).
3. Camus, C. et al. Scaling up bioethanol production from the farmed brown macroalga Macrocystis pyrifera in Chile. Biofuels, *Bioproducts and Biorefining* 10, 673–685 (2016).
4. Contador, C. A. et al. Analyzing redox balance in a synthetic yeast platform to improve utilization of brown macroalgae as feedstock. *Metabolic Engineering Communications* 2, 76–84 (2015).